ROLE OF MACROPHAGES IN THE POLYCLONAL AND SPECIFIC RESPONSE TO T-INDEPENDENT ANTIGEN IN VITRO

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Data on the effect of adhesive cells (macrophages) on the T-independent immune response have recently been published, but different authors have noted differences in the degree of this dependence [5-7, 10]. An important feature of these experiments is comparison of the specific immunizing and polyclonal action of T-independent antigens, for the second type of action is very strong. These two effects may depend to different degrees on the presence of macrophages. In experiments in vitro, the objective parameters of the immunizing and polyclonal action of the antigen are, in particular, the levels of specific antibodies (Ab) and of total immunoglobulins (Ig) in the culture medium of lymphocytes.

The aim of this investigation was to study in vitro the role of macrophages in the response of mouse spleen cells to Salmonella typhi Vi antigen. The necessary modifications were made to the method of enzyme immunoassay used for quantitative evaluation of Ab.

EXPERIMENTAL METHOD

The primary response to Vi antigen was produced in a culture of spleen cells from male CBA mice. The conditions of culture were described previously [3]. The concentration of Vi antigen was 25 μg/ml. The splenocyte culture contained either the whole pool of spleen cells or cells freed from the majority of adherent cells by adsorption on a plastic dish. The number of cells in the sample (250 μl) was 5×10^5.

At the end of the culture time (96 h) the content of total Ig and of specific Ab against Vi antigen (Vi-Ab) was determined in the supernatant of the culture fluid by the ELISA technique [2]. Total Ig were assayed by the usual method [11]. Polystyrene microtitration panels (made by the All-Union Research Institute of Medical Engineering, Ministry of the Medical Industry of the USSR) wer sensitized with rabbit immune Ig isolated from the serum of animals immunized with Ig of CBA mice. Next the test samples were introduced into the wells and the reference preparation of mouse Ig titrated simultaneously. After incubation and washing, a conjugate of rabbit antimouse Ig with peroxidase was applied to the micropanel, and last of all, after incubation and washing, the substrate for the enzyme, a solution of orthophenylenediamine, was added. The enzyme reaction was recorded on an automatic photometer at 492 nm.

The immune conjugate of peroxidase was prepared by the method in [12] by a combination of rabbit Ig, isolated from immune serum against mouse Ig by precipitation with ammonium sulfate at 35% saturation, and of peroxidase (from Sigma, USA). The working dilution of the conjugate was 1:1500.

Determination of specific Vi-Ab by ELISA showed that normal mouse serum in neutral medium was adsorbed only 4 times less strongly than specific anti-Vi-serum on micropanels sensitized with Vi antigen (Fig. 1). This state of affairs would interfere with the correct quantitative estimation of Ab in the culture fluid. A significant decrease in nonspecific adsorption was obtained by lowering the reaction of the medium in the stage of application of the samples and reference antiserum to pH 3.5 (Fig. 1), whereas addition of detergent [4, 11] or preliminary isolation of the γ-globulin fraction from the serum, which is usually done for this purpose, did not have the required effect.

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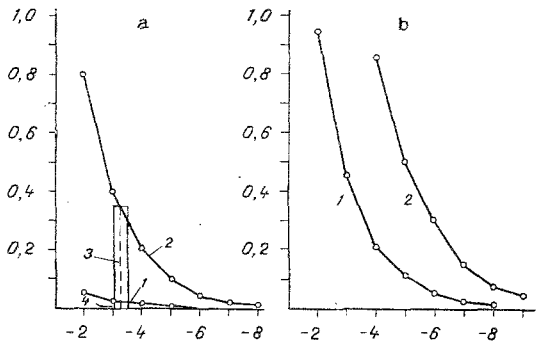

Fig. 1. Adsorption of normal and immune anti-Vi-sera from CBA mice on micropanels sensitized with Vi antigen at pH 3.5 (a) and 7.2 (b). Abscissa, $\log_2$ of 100-fold dilution of serum; ordinate, extinction of samples at wavelength of 492 nm. 1) Normal serum; 2) anti-Vi-serum. Level of adsorption of aliquots from supernatant of splenocyte culture stimulated by Vi antigen (3) and of culture medium (4) is shown. Levels of Ig and Ab, synthesized in the initial culture, and not subjected to adsorption on plastic, taken as 100%.

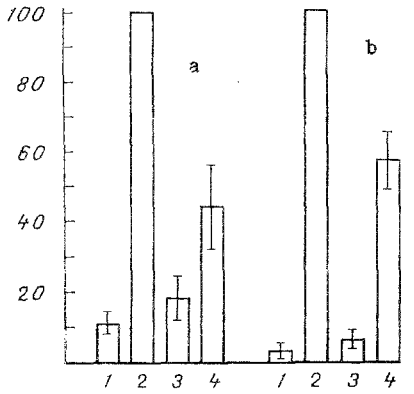

Fig. 2. Level of total (a) and specific Vi-Ab (b) in cultures of splenocytes (in %). 1) Levels of Ig and Ab in original splenocyte culture in absence of Vi antigen; 2) levels of Ig and Ab in splenocyte culture stimulated by Vi antigen (taken as 100%); 3) levels of Ig and Ab in splenocyte culture freed from adherent cells by adsorption on plastic, in absence of antigen; 4) levels of Ig and Ab in splenocyte culture freed from adherent cells and stimulated by Vi antigens.

To analyze Vi-Ab the microtitration panels were first sensitized with Vi antigen (1 μg per well in 100 μl of 0.15 M NaCl solution, pH 7.2). After incubation overnight at 4°C the unbound antigen was removed by washing the panels with water and with a solution of Tween-20, after which aliquots of the test samples were introduced into the wells in a 1% solution of bovine serum albumin, made up in 0.1 M citrate buffer, pH 3.5, containing 0.05% Tween-20. Immune anti-Vi-serum from CBA mice was titrated in the same medium as the reference preparation. The panels were incubated for 2.5 h at room temperature and washed, after which the immune peroxidase conjugate was added. The subsequent analysis proceeded as in the case of total Ig.

Dilutions of immune anti-Vi-serum corresponding to the concentrations of Vi-Ab in the test samples were found by using the established logarithmic relationship (Fig. 1), and after correction for the control, their Ab titer was determined. The concentration of Vi-Ab in the immune serum was determined beforehand by the quantitative precipitation test [8]. The results given below are mean data of analysis of seven spleen cell cultures.

EXPERIMENTAL RESULTS

When medium with pH 3.5 was used the level of specific Vi-Ab synthesized during incubation for 96 h in a Vi-antigen-stimulated lymphocyte culture, was 65 ± 19 ng/ml of culture. Meanwhile the level of total Ig synthesized under these conditions reached 3967 ± 1071 ng/ml. Thus the fraction of specific Vi-Ab in the antigen-stimulated culture did not exceed 2% of the total synthesized Ig. This is in agreement with results obtained by the study of the dynamics of Ab-forming cells and of cells synthesizing nonspecific Ig in the spleen of mice immunized with Vi antigen [1].

The level of total Ig is an unstimulated splenocyte culture was 583 ± 146 ng/ml culture fluid.

The results of determination of levels of total Ig and specific Vi-Ab, induced by Vi antigen in a splenocyte culture, freed from most macrophages by adsorption on a plastic dish, are given in Fig. 2. Removal of the adherent cells (macrophages) from a Vi antigen-stimulated cell culture caused a decrease in the synthesis of both nonspecific Ig and Vi-Ab.

The role of macrophages in inducing the formations of cells synthesizing nonspecific Ig was demonstrated previously, indirectly – with the aid of antiserum to leukocytic pyrogen (interleukin 1). It was found that this antiserum, which binds the product of activated macrophages, inhibits lipopolysaccharide (LPS)-induced conversion of B lymphocytes into Ig-synthesizing cells [9]. According to other data [5], thorough purification of a suspension of B lymphocytes from T cells and macrophages prevents the B cells from responding to the activating action of LPS.

The data in this paper add to the picture of the role of macrophages in the T-independent immune response: removal of 90% of the macrophages from the population leads to a marked decrease in the ability of B lymphocytes to respond to the polyclonal and immunizing action of Vi antigen. The presence of macrophages is therefore essential for realization of both types of action to an equal degree.

It is also clear from Fig. 2 that the background Ig level in the splenocyte culture is not lowered after removal of the adherent cells. Hence it follows that the role of macrophages in the T-independent immune response is most important at the stage of lymphocyte activation.

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